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Effects of various light-emitting diode wavelengths on periodontopathic bacteria and gingival fibroblasts: An *in vitro* study

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ABSTRACT

Background: In recent years, light has been used for bacterial control of periodontal diseases. This *in vitro* study evaluated the effects of light-emitting diode (LED) irradiation at different wavelengths on both *Porphyromonas gingivalis* and human gingival fibroblasts (HGF-1).

Methods: P. gingivalis suspension was irradiated with LEDs of 365, 405, 450, 470, 565, and 625 nm at 50, 100, 150, and 200 mW/cm^2 for 3 min (radiant exposure: 9, 18, 27, 36 J/cm^2, respectively). Treated samples were anaerobically cultured on agar plates, and the number of colony-forming units (CFUs) was determined. Reactive oxygen species (ROS) levels were measured after LED irradiation. The viability and damage of HGF-1 were measured through WST-8 and lactate dehydrogenase assays, respectively. Gene expression in *P. gingivalis* was evaluated through quantitative polymerase chain reaction.

Results: The greatest reduction in *P. gingivalis* CFUs was observed on irradiation at 365 nm with 150 mW/cm^2 for 3 min (27 J/cm^2), followed by 450 and 470 nm under the same conditions. While 365-nm irradiation significantly decreased the viability of HGF-1 cells, the cytotoxic effects of 450- and 470-nm irradiation were comparatively low and not significant. Further, 450-nm irradiation indicated increased ROS production and downregulated the genes related to gingipain and fimbriae. The 565- and 625-nm wavelength groups exhibited no antibacterial effects; rather, they significantly activated HGF-1 proliferation.

Conclusions: The 450- and 470-nm blue LEDs showed high antibacterial activity with low cytotoxicity to host cells, suggesting promising bacterial control in periodontal therapy. Additionally, blue LEDs may attenuate the pathogenesis of *P. gingivalis*.

1. Introduction

Periodontal disease is an inflammatory condition characterized by destruction of tooth-supporting tissues. Although the disease progression can be influenced by various environmental elements, as well as host systemic conditions, bacterial plaque is considered the primary etiological factor [1], and plaque control through reinforcement of oral hygiene instructions and professional mechanical plaque removal is performed as the first step in the management of periodontal disease. However, conventional periodontal therapies cannot completely eradicate bacteria from periodontal pockets and root surfaces with complex morphology [2,3]. To reinforce the elimination of bacteria, antibiotics are accordingly administered as an adjunct to mechanical plaque debridement; however, they also have disadvantages, such as allergy, presence/emergence of drug-resistant bacteria, and difficulty in maintaining optimal drug concentrations in periodontal pockets [4].

Recently, the application of light energy has been identified as a new therapeutic method for controlling bacterial infections in periodontal diseases [5–7]. Light-emitting diode (LED) is a non-coherent light, and has advantages over lasers, such as the ability to irradiate a large area

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with less invasiveness, lower cost, and smaller size of the equipment. These characteristics suggest that phototherapy using LED may be useful for periodontal care in both professional and home settings. Several studies have used LEDs as the light source for antimicrobial photodynamic therapy (aPDT) to inactivate pathogenic bacteria and suppress periodontal inflammation [8]. Photodynamic reactions of aPDT occur upon light irradiation of photosensitizers in the presence of oxygen [9], resulting in the production of reactive oxygen species (ROS) that damage diverse bacterial structures, thereby inducing an antibacterial effect. Moreover, a specific wavelength of LED light is known to suppress some periodontopathic bacteria by irradiation alone in the absence of exogenous photosensitizers [10–13]. However, till date, only few studies have compared the antibacterial effects of various wavelengths of LED light. The experimental conditions across these previous studies were not uniform, and a precise comparison of their results is difficult. Moreover, some wavelengths of light can activate host cells and, conversely, they may also promote bacterial growth [14,15]. Thus, it is necessary to investigate the appropriate conditions of irradiation time and power of LEDs, as well as the selection of wavelengths for the application of LEDs alone in clinical practice.

In the present *in vitro* study, we evaluated the effects of various LED light wavelengths on *Porphyromonas gingivalis*, a major periodontal pathogen associated with periodontitis, and gingival fibroblasts under the same irradiation conditions.

2. Materials and methods

2.1. Light sources

A high-power LED controller and mounted LEDs of various wavelengths were used (DC2200; Thorlabs Inc., Newton, NJ, USA): ultraviolet A light (UVA; peak wavelength: 365 nm), blue-violet light (405 nm), blue light (450 and 470 nm), yellow-green light (565 nm), and red light (625 nm). During irradiation, the irradiances were measured above the well using a power meter (PM100D; Thorlabs Inc.). The irradiations were performed one well at a time, from the bottom of each well (6.8 mm in diameter), with irradiances of 50, 100, 150, and 200 mW/cm^2 for 3 min (radiant exposure: 9, 18, 27, and 36 J/cm^2, respectively).

2.2. Preparation of bacterial suspension

P. gingivalis (ATCC 33277) obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA) was maintained on a trypticase soy blood agar plate [16] under anaerobic conditions. A loopful (1.0 mm in diameter) of bacteria was taken from the plate and anaerobically cultured in 9 mL of brain heart infusion medium (Difco Laboratories, Franklin Lakes, NJ, USA) supplemented with 5 mg/L of hemin (Fujifilm Wako Pure Chemical Industries, Osaka, Japan) and 1 mg/L of vitamin K1 (KOA ISEI Co., Ltd., Yamagata, Japan) at 37°C. Immediately before the experiment, the bacterial suspension in the mid-log phase was diluted to adjust the concentration to 1×10^8 cells/mL using a counting chamber. After centrifugation of the bacterial suspension, the supernatant was removed and replaced with the same volume of 0.9% w/v sodium chloride (NaCl) solution for resuspension.

2.3. Culture of human gingival fibroblasts (HGF-1)

HGF-1 (cell line ATCC CRL-4061) obtained from ATCC were cultured in Dulbecco's modified Eagle medium (Fujifilm Wako Pure Chemical Industries) supplemented with 10 % fetal bovine serum (Gibco, Carlsbad, CA, USA) and 1 % antibiotic antimycotic mixture (Fujifilm Wako Pure Chemical Industries) in a humidified atmosphere with 5 % carbon dioxide at 37°C. HGF-1 were seeded at 1.0×10^4 cells per well in 96-well flat-bottom plates (Falcon®, Corning Inc., Corning, NY, USA). Forty-eight hours after seeding, the medium was removed, and 200 μL of 0.9% w/v NaCl was added.

2.4. Antibacterial effects of various wavelengths of LED on P. gingivalis

Two hundred microliters of *P. gingivalis* suspension was added to each well of a sterile 96-well flat-bottom plate. LED irradiation at different wavelengths (365, 405, 450, 470, 565, and 625 nm), with irradiances of 50, 100, 150, and 200 mW/cm^2, was performed for 3 min from the bottom of each well under aerobic conditions (radiant exposure: 9, 18, 27, and 36 J/cm^2, respectively). After 3 min of irradiation, the bacterial suspensions were serially diluted with 0.9% w/v NaCl solution and plated in triplicate onto trypticase soy blood agar plates. They were incubated anaerobically at 37 °C for 1 week, and the colony forming units (CFU) were calculated.

2.5. Viability of HGF-1 following LED irradiation with different wavelengths

HGF-1 were irradiated with different LED wavelengths (365, 405, 450, 470, 565, and 625 nm) at 27 J/cm^2 (150 mW/cm^2 for 3 min). The viability of HGF-1 was measured using the WST-8 (Cell Counting Kit-8, Dojindo, Kumamoto, Japan) and lactate dehydrogenase (LDH) assays (Cytotoxicity Detection Kit, Roche, Mannheim, Germany) according to the manufacturer's instructions on Day 1 and Day 3 following treatment. The optical absorbance of the samples was measured using a fluorescence microplate reader at 450 and 490 nm (VMAX, Molecular Devices, Sunnyvale, CA, USA). The ratio of the cell viability of irradiated HGF-1 cells relative to that of the untreated control was calculated using the WST-8 assay. The relative cytotoxicity was expressed as the ratio of the LDH activity in irradiated cells to that in nonirradiated cells. The LDH assay for each sample dish was performed in duplicate, and the mean value was used for statistical analyses.

2.6. Measurement of total ROS on P. gingivalis

Intracellular ROS were detected using a commercially available kit (ROS Assay Kit-Highly Sensitive 2′, 7′dichlorofluorescin diacetate [DCFH-DA]; Dojindo), according to the manufacturer's instructions. A working solution of highly sensitive DCFH-DA dye was added to the *P. gingivalis* pellet to make a *P. gingivalis* suspension. Two hundred microliters of *P. gingivalis* suspension was added to each well of a sterile 96-well flat-bottom plate. The *P. gingivalis* suspension was irradiated with LEDs at different wavelengths (365, 450, and 625 nm) at 150 mW/cm^2 for 30 s, 1 min, or 3 min (radiant exposure: 4.5, 9, or 27 J/cm^2, respectively). After the irradiation, the *P. gingivalis* suspension was incubated anaerobically at 37 °C for 30 min. The suspensions were washed three times with sterile saline solution, and the changes in the relative fluorescence intensities of ROS at 485 and 520 nm were detected using a plate reader (FLUOstar® OPTIMA-6, BMG Labtech Japan Ltd., Saitama, Japan).

2.7. RNA quality and gene expression profiling of P. gingivalis following LED irradiation

The *P. gingivalis* suspension was irradiated with LEDs (450 and 625 nm) at 150 mW/cm^2 for 3 min (radiant exposure: 27 J/cm^2) and was then mixed with RNA protect™ Bacteria Reagent (Qiagen, Tokyo, Japan) to stabilize the bacterial RNAs. Bacteria were disrupted using a FastPrep® FP120 Instrument (QBiogene, Carlsbad, CA, USA), and total RNAs were isolated using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA) and a PureLink® RNA Nano Kit (Invitrogen), according to the manufacturer's instructions. For quantitative measurement of RNA degradation, the RNA integrity number [17] was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) with an RNA 6000 Nano LabChip® Kit (Agilent Technologies). Reverse transcription was performed using random hexamers (Invitrogen) and Superscript II Reverse Transcriptase (Invitrogen). Quantitative polymerase chain reaction was performed using a Thermal Cycler Dice® Real

Time System III (Takara, Shiga, Japan) with SYBR Premix Ex Taq™ (Takara) as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 30 s. The primers used are listed in Table 1 [12,18,19]. All data were analyzed based on the comparative threshold cycle method [20].

2.8. Statistical analysis

Data are presented as mean ± standard deviation. Since a Shapiro-Wilk test indicated that the data were consistent with a normal distribution, one-way analysis of variance followed by Tukey's HSD post hoc test was performed to compare the bacterial count (Fig. 1 and 2), viability of HGF-1 (Fig. 3 and 4), ROS induction level (Fig. 5), and gene expression level (Fig. 6) in each group. All statistical analyses were performed using a statistical software program (RStudio version 1.2.5001; Boston, MA, USA).

3. Results

3.1. Antibacterial effects of LED on P. gingivalis at various wavelengths

The effect of LED irradiation on *P. gingivalis* was evaluated at different wavelengths (365, 405, 450, 470, 565, and 625 nm) (Fig. 1). The CFU counts following LED irradiation at 365, 405, 450, and 470 nm decreased with increasing irradiances, whereas the CFU counts in the 565- and 625-nm wavelength groups did not differ from those in the control, even at the highest irradiance (36 J/cm^2). Under the same irradiation conditions [27 J/cm^2 (150 mW/cm^2, 3 min)], the greatest reduction in CFUs compared to the untreated control was observed in the 365-nm (3.6 log reduction) wavelength group, followed by the 450-nm (2.2 log reduction) and 470-nm (1.8 log reduction) wavelength groups (Fig. 2).

3.2. Effects of LED irradiation with different wavelengths on HGF-1 viability

The viability of HGF-1 on Day 1 and Day 3 following LED irradiation at different wavelengths is shown in Fig. 3. On Day 1, 365-nm irradiation significantly reduced the viability of HGF-1 (81.5 %) compared to that in the control. Although a slight reduction in viability was also observed in the 450-nm and 470-nm wavelength groups (91.2 % and 91.6 %, respectively), the viability in these groups did not differ from that in the control. In contrast, an increase in viability was observed in the 565-nm and 625-nm wavelength groups (114.1 % and 109.1 %, respectively), although there was no significant difference compared with the viability in the control.

On Day 3, a significant reduction in viability was observed in the 365-nm and 405-nm wavelength groups (58.2 % and 72.8 %, respectively), and the 450- and 470-nm wavelength groups showed further reduction (87.9 % and 84.1 %, respectively); however, there was no significant difference compared with the viability in the control. The viability of the 565- and 625-nm wavelength groups had increased further (127.1 % and 132.5 %, respectively), with significant differences compared with that in the control. There was no significant difference in the LDH activity of the control and all irradiated groups on Day 1 and Day 3 (Fig. 4). The results suggest that the LEDs exerted an antibacterial

Table 1
PCR primers used in this study.

Gene name (Gene ID[a])	Direction[b]	Primer sequence (5′–3′)	Size (bp)	Description	Reference[c]
dnaA	F	TTTGGAGGGCAATTTCGTAG	124	Chromosome replication initiator	[12]
(PGN_0001)	R	TGTCACCGTACCGGGATATT			
dnaB	F	TGCCGATATGGTATGCTTCA	138	Replicative DNA helicase	[12]
(PGN_1378)	R	CGCAAACGTACATCATCCAC			
dnaG	F	TGTGCAGAAGTTCCAACTCG	136	DNA primase	[12]
(PGN_1751)	R	TAAGGCCTTGCTGTCTTCGT			
dnaE	F	GCAATCGTTTAGCCAAGCTC	132	DNA polymerase III α submit	[12]
(PGN_0034)	R	GGGTATCGCGCATTACCTTA			
recA	F	GAATGGCCACGGAGAAGATA	137	DNA repair	[12]
(PGN_1057)	R	GTAACCGCCTACACCGAGAG			
polA	F	GAGACCGACTCGAAAGATGC	137	DNA polymerase I	[12]
(PGN_1771)	R	AGCGGACGCAAGAGATCTAA			
htpG	F	AATGGAAAGACGGCAAGATG	91	Heat shock protein 90	[12]
(PGN_0041)	R	TTGAGGTCAGCAGGCTTTTT			
ftsH	F	GTAGGAGCCTCTCGTGTTCG	132	Cell division protein	[12]
(PGN_0043)	R	CTCATCATTGCCGGAGAAAT			
clpB	F	AGAAACTGCCCCATGTATCG	129	Component of the stress response protein	[12]
(PGN_1208)	R	CTAGCACGATGTGCTCCAAA			
hbp35	F	TCTTGCTTTCGTACACCATC	93	35 kDa hemin binding protein	
(PGN_0659)	R	GCTGTAGGCTCTGCTTTATC			
sulA	F	CGCTGGGAGATCGTTACATT	101	Capsular polysaccharide biosynthesis protein	[12]
(PGN_1525)	R	GGGGGAAAGACCTTTGAATC			
ftn	F	AGCACGCCTACGATATGA	100	Ferritin	
(PGN_0604)	R	TCCAATACAGAGCCGAACT			
rgpA	F	GCCGAGATTGTTCTTGAAGC	256	Arginine-specific cysteine proteinase RgpA	[19]
(PGN_1970)	R	AGGAGCAGCAATTGCAAAG			
rgpB	F	CCTACGTGTACGGACAGAGCTATA	70	Arginine-specific cysteine proteinase RgpB	[18]
(PGN_1466)	R	AGGATCGCTCAGCGTAGCATT			
kgp	F	AGCTGACAAAGGTGGAGACCAAAGG	186	Lysine-specific cysteine proteinase Kgp	[19]
(PGN_1728)	R	TGTGGCATGAGTTTTTCGGAACCGT			
fimA	F	CAGCAGGAAGCCATCAAATC	140	Structural subunit of the major fimbriae	[19]
(PGN_0180)	R	CAGTCAGTTCAGTTGTCAAT			
fimC	F	AATGAACCCGATGCCCTTAC	82	Minor component of fimbriae	
(PGN_0183)	R	TCACATCAGGAGCTTGCATATC			
fimD	F	AGCAACCACCTGTACGATATG	93	Minor component FimD	
(PGN_0184)	R	CACAGAGAGAGAGCCGTTTATC			

[a] Locus number from NCBI (http://www.ncbi.nlm.nih.gov/nuccore/NC_010729.1).
[b] F, forward. R, reverse.
[c] (blank), Original design in this study.

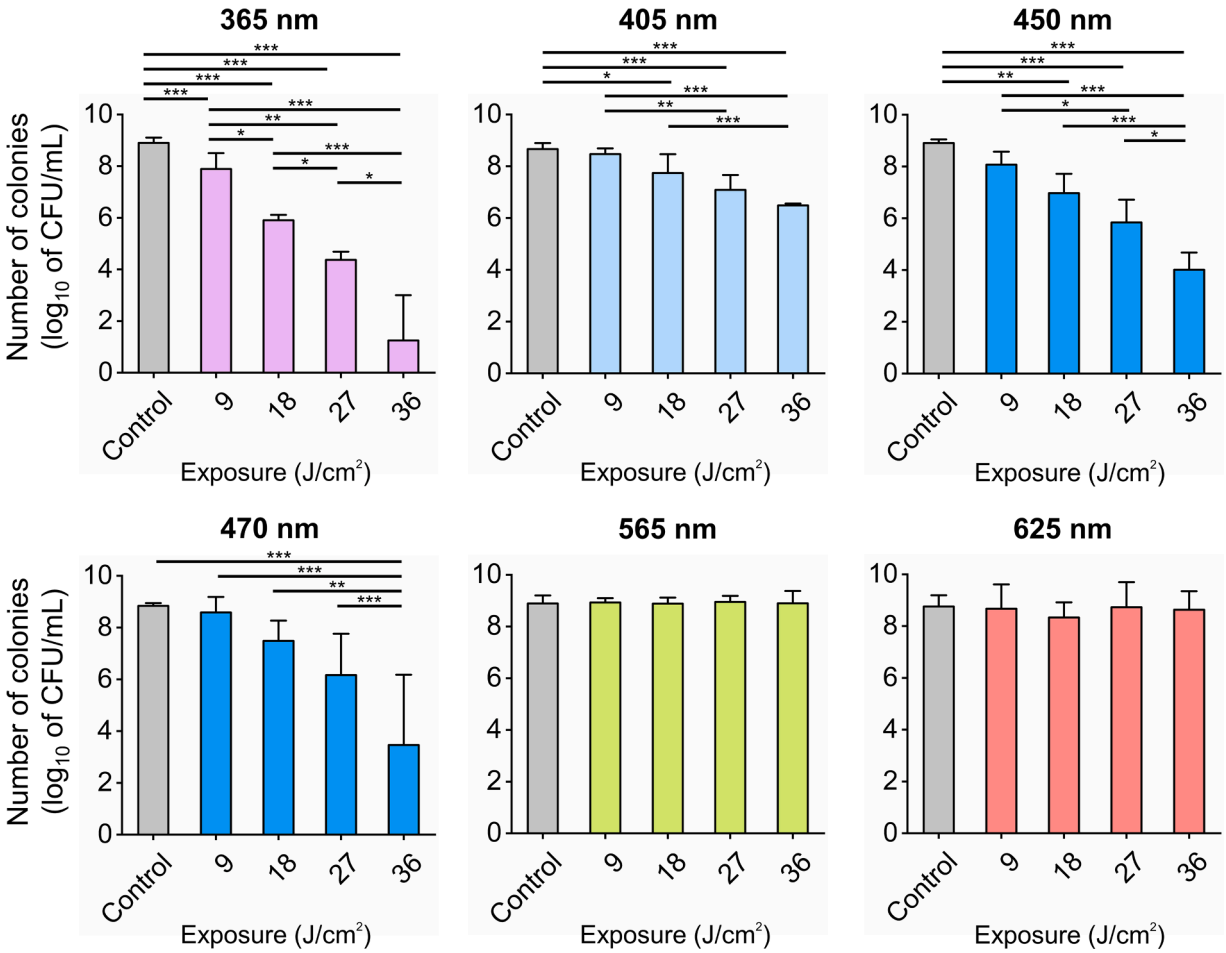

Fig. 1. Antibacterial effect of various wavelengths of LED on *Porphyromonas gingivalis*: comparison at different irradiances for each wavelength. *P. gingivalis* suspension was irradiated with 365-, 405-, 450-, 470-, 565-, and 625-nm LED. *P. gingivalis* suspension without irradiation was used as a control. Data are presented as the mean ± standard deviation of four independent experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (Tukey's HSD test). CFU, colony-forming units.

effect mainly with short wavelengths (365, 450, and 470 nm). Together with the results of the viability test of HGF-1, we focused on blue LEDs (especially 450 nm) and investigated their antibacterial effects.

3.3. Total ROS induction in P. gingivalis following LED irradiation

The ROS levels of *P. gingivalis* were measured after irradiation with LEDs (365, 450, and 625 nm) at 4.5 J/cm^2 (150 mW/cm^2, 30 s), 9 J/cm^2 (150 mW/cm^2, 1 min), and 27 J/cm^2 (150 mW/cm^2, 3 min. At 4.5 J/cm^2 and 9 J/cm^2, the amount of ROS generated by the 450-nm group was greater than that of the 365- and 625-nm groups, and approximately 1.1–1.2 times higher than that of the control group, although there was no significant difference (data not shown). The amount of ROS generated with 450-nm LED at 27 J/cm^2 was the highest among the three groups and was 1.4 times that in the control. Further, the 365-nm LED induced a significantly higher ROS, which was 1.2 times higher than that in the control. The ROS level after 625-nm irradiation was similar to that in the control (Fig. 5).

3.4. RNA quality and gene expression profiling of P. gingivalis following LED irradiation

Gene expression of *P. gingivalis* following 450- and 625-nm irradiation was examined (Fig. 6). Irradiation with 450-nm wavelength decreased the expression of many genes, including genes related to chaperones (*htpG* [PGN_0041] and *clpB* [PGN_1208]), SOS response (*recA* [PGN_1057]), DNA replication (*polA* [PGN 1771]), *P. gingivalis* specific protease (gingipains) (*rgpB* [PGN_1466], *kgp* [PGN_1728], and *rgpA* [PGN_1970]), and structures on the bacterial surface (fimbriae) (*fimA* [PGN_0180], *fimC* [PGN_0183], and *fimD* [PGN_0184]); the expression of hemin binding protein (*hbp35* [PGN_0659]) reduced significantly compared with that in the control. Further, in the 625-nm wavelength group, expression of *recA* and *fimA* was significantly reduced, but genes related to cell division (*ftsH* [PGN_0043]), iron-binding protein [*ftn* (PGN 0604)], and *polA* were upregulated.

4. Discussion

In this study, we evaluated the effects of various LED irradiation wavelengths on *P. gingivalis* and HGF-1 under identical irradiation conditions. Several studies have shown the phototoxic effects of blue LED without exogenous photosensitizers on periodontal bacteria, including *P. gingivalis* [10,21–23]. We previously reported that ultraviolet and blue LEDs have antibacterial effects against *P. gingivalis* [9,12,13,24]. In the present study, irradiation with UVA (365 nm), blue-violet (405 nm), and blue (450 and 470 nm) LEDs reduced the number of *P. gingivalis* cells, which is consistent with previous reports. The antibacterial effect increased in an irradiance-dependent manner. Aung et al. reported the absence of any antimicrobial effect of UVA (365 nm) and blue (448 nm) LEDs at low irradiance levels (600 mJ/cm^2) against *P. gingivalis* [13]. The radiant exposure of LED light in this study was approximately four times higher than that used in their study, and we considered that the disparity in light exposure could be the primary reason for the antibacterial effect observed in our study. In other words,

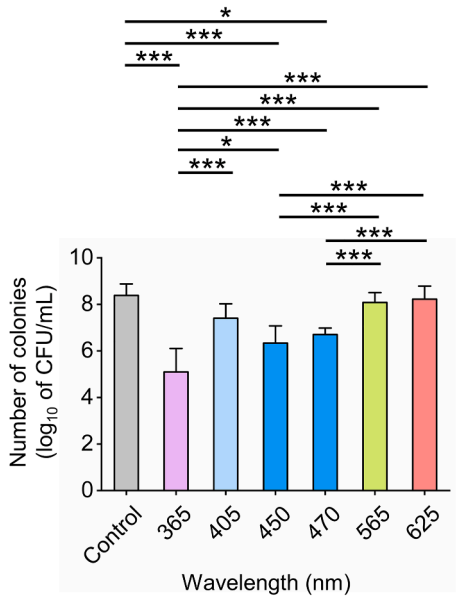

Fig. 2. Antibacterial effect of various wavelengths of LED on *Porphyromonas gingivalis*: comparison at the same irradiance among wavelengths. *P. gingivalis* suspension was irradiated with 365-, 405-, 450-, 470-, 565-, and 625-nm LED at 27 J/cm^2 (150 mW/cm^2, 3 min). *P. gingivalis* suspension that underwent no irradiation was employed as control. Data are presented as the mean ± standard deviation of four independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Tukey's HSD test). CFU, colony-forming units.

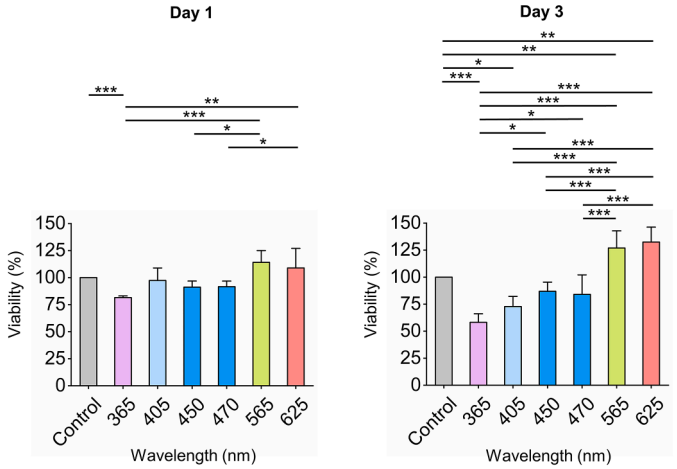

Fig. 3. Viability of human gingival fibroblasts (HGF-1) following LED irradiation with different wavelengths at the same irradiance. Relative proliferation of cells by WST-8 assay on Day 1 and Day 3 after irradiation [27 J/cm^2 (150 mW/cm^2, 3 min)] compared to the nonirradiated control cells. Data are presented as the mean ± standard deviation of four independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Tukey's HSD test).

a certain threshold of light energy would be required to induce an antibacterial effect. For the clinical application of LED irradiation in periodontal treatment, the high antibacterial effects must be accompanied with minimal damage to host cells. Although in our preliminary study, UVA and blue light exerted strong antibacterial effects after irradiation at 36 J/cm^2 (200 mW/cm^2, 3 min), high cytotoxicity on HGF-1 was also observed; HGF-1 viability in the 365-, 405-, and 450-nm wavelength groups on Day 3 was reduced to 50.3 %, 61.5 %, and 65.3 %, respectively. Following the results of preliminary study, we investigated the effects of LED irradiation on *P. gingivalis* and HGF-1 cells at 27 J/cm^2 (150 mW/cm^2, 3 min) with lower cytotoxicity.

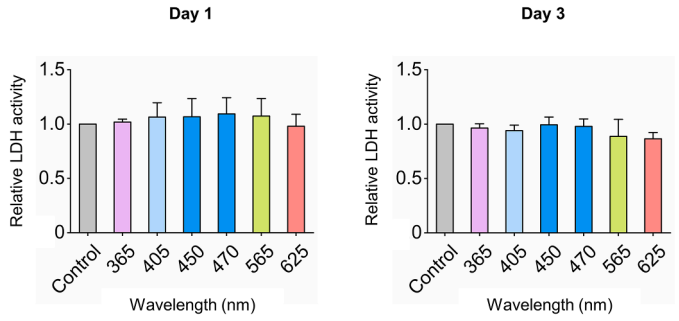

Fig. 4. Lactate dehydrogenase (LDH) activity of human gingival fibroblasts (HGF-1) following LED irradiation with different wavelengths at the same irradiance. Relative LDH activity in the cultured medium of HGF-1 on Day 1 and Day 3 after irradiation [27 J/cm^2 (150 mW/cm^2, 3 min)] compared to that of nonirradiated control cells. Data are presented as the mean ± standard deviation of four independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Tukey's HSD test).

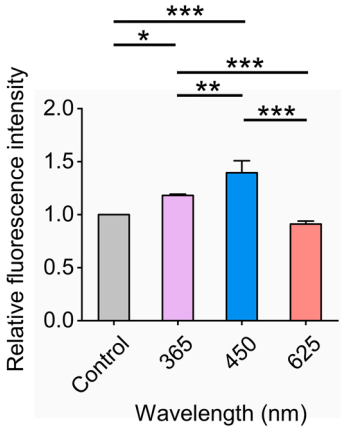

Fig. 5. Total reactive oxygen species (ROS) in *Porphyromonas gingivalis* following 365-, 450-, and 625-nm LED irradiation. Data are presented as the mean ± standard deviation of four independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Tukey's HSD test).

Iron is an essential nutrient for many species of bacteria to survive inside mammalian hosts. *P. gingivalis* degrades hemoglobin to derive heme, a complex of iron and the tetrapyrrole protoporphyrin IX [25], and endogenous porphyrin is produced when the bacterium further acquires iron from heme. Porphyrin as a photosensitizer in bacterial cells can absorb light and generate ROS, leading to disruption of the bacterial cell membrane and apoptosis [10,26,27]. Yoshida et al. have confirmed that the action of aPDT is mediated by endogenous protoporphyrin IX within *P. gingivalis* bacteria subsequent to irradiation with 460-nm LED light [27]. The present study also found a significant increase in ROS production after 450-nm light irradiation, suggesting that the antibacterial action of the blue light is related to ROS generation, which is associated with endogenous porphyrins in *P. gingivalis*. In contrast, the antibacterial effect and cytotoxicity of UVA (365 nm) were higher than those of blue light (450 and 470 nm). These results suggest that UVA exerts its effects not only through ROS production but also by other mechanisms, such as alteration of gene expression by photochemical reactions. However, it remains controversial whether UVA induces DNA damage and represses RNA synthesis like UVC (260 nm).

On the other hand, no antibacterial effects were observed with yellow-green (565 nm) or red (625 nm) LED irradiation. This is consistent with previous findings on the minimal antibacterial effects of a single application of yellow-green and red LED lights [28,29–31]. Although the radiant exposure used in this study (maximum 36 J/cm^2)

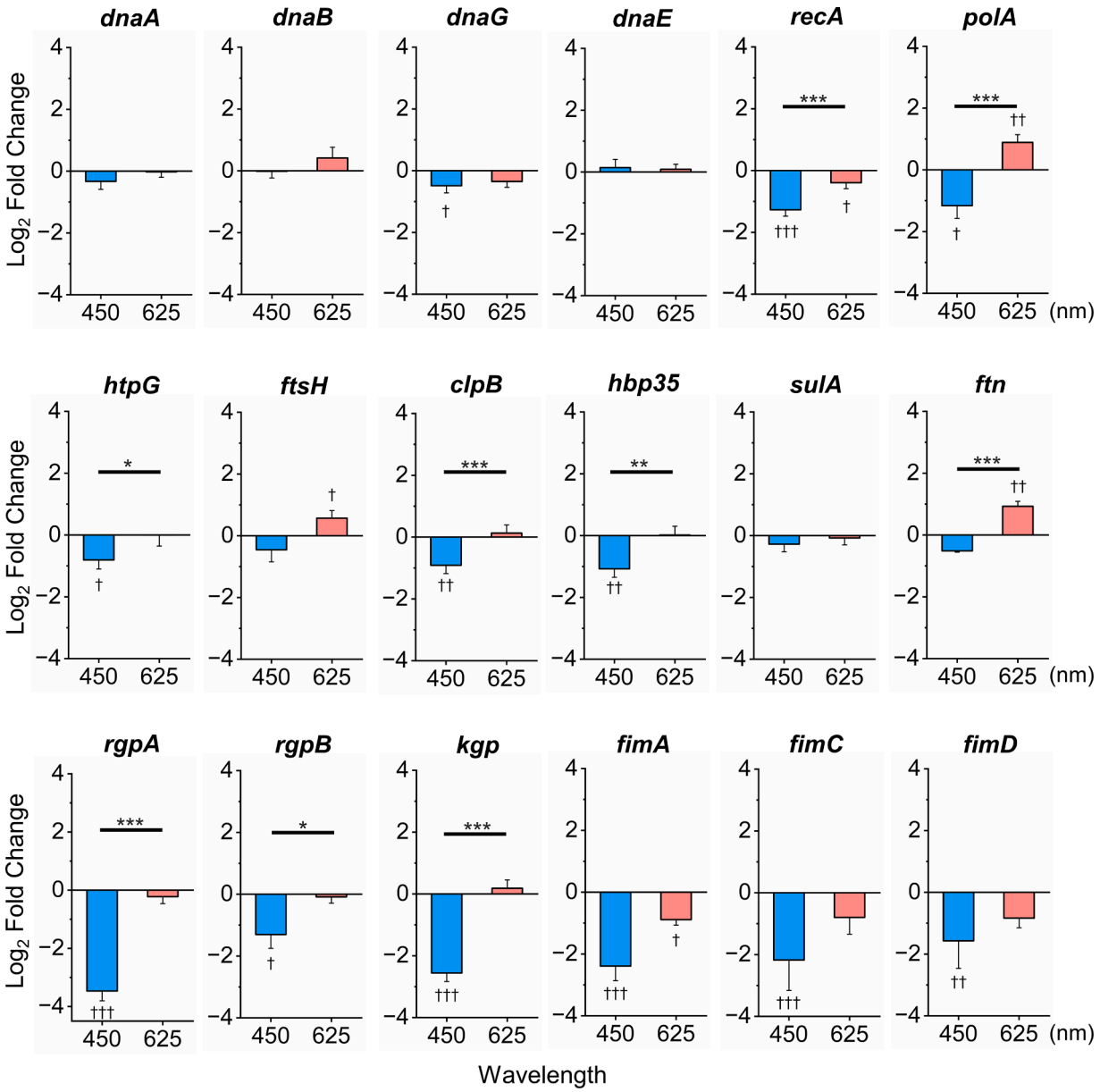

Fig. 6. Gene expression profiling of *Porphyromonas gingivalis* following 450- and 625-nm LED irradiation. The fold change [$\log_2$ fold change (*vs*. control)] in gene expression was calculated based on the comparative threshold cycle method. Data are presented as the mean ± standard deviation of four independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (450 nm *vs* 625 nm) $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ (*vs* control) (Tukey's HSD test).

was much higher than that used in previous studies by Kitanaka et al. (565 nm, 8.56 J/cm^2) [28] and Moslemi et al. (625 nm, 6 J/cm^2) [30], we still could not observe any antibacterial effect. Unlike the UVA and blue LEDs, red LED might promote the growth of *P. gingivalis*. Kim et al. reported that the viability of *P. gingivalis* increased after 8 h irradiance at 625 nm LED (172.8 J/cm^2) compared to the non-irradiated control [15]. *P. gingivalis* proliferation was not observed in the present study; however, HGF proliferation following 565- or 625-nm LED irradiation was observed. Vinck et al. showed that green (570 nm) and red (660 nm) LED irradiation increased *in vitro* fibroblast proliferation [32]. Low-dose irradiation at a specific wavelength is thought to stimulate and activate host cell function. For example, LED irradiation at different wavelengths in the range of 390–600 nm with low-radiant exposure of 0.04–50 J/cm^2 has been reported to stimulate healing and restore skin function [33].

The oxidative stress caused by ROS damages bacterial cell membranes, proteins, and genes [27,34–36]. Gene expression was analyzed to compare the effects of blue (450 nm) and red (625 nm) LED on *P. gingivalis*. We previously reported that 470-nm irradiation significantly decreased the expression of genes related to DNA replication and cell division [12]. In the present study, the expression of a gene related to DNA replication (*polA*), as well as genes related to chaperones (*htpG* and *clpB)* and the SOS response (*recA*), was reduced after 450 nm irradiation. Molecular chaperones promote protein folding and prevent protein misfolding and aggregation [37]. The SOS response in bacteria is a global response to DNA damage, in which the cell cycle is arrested, and DNA repair and mutagenesis are induced [38]. Our results suggest that 450-nm LED irradiation may decrease the broad general stress response during the stationary phase of *P. gingivalis*.

Gingipains are the major proteolytic enzymes of *P. gingivalis* and act as virulence factors in the development of periodontitis. Iron is essential for the growth of *P. gingivalis*, and gingipains are also closely linked to heme/iron acquisition from erythrocytes, promoting bacterial survival and proliferation [39,40]. In the present study, genes related to

gingipains (*rgpA, rgpB, kgp*) and hemin binding protein 35 (*hbp35*), which is related to hemin acquisition, were downregulated. These results indicate the inhibition of *P. gingivalis* growth and pathogenicity by blue LED irradiation. Yuan et al. recently reported that irradiation with 405-nm light at 30 J/cm^2 (100 mW/cm^2, 5 min) led to significant upregulation of *rgpA* and *rgpB*, as well as that of *ftn* and *fetB* [41]. This discrepancy may be attributed to the differences in the bacterial and light irradiation conditions used in the studies. It is also possible that even slight differences in light wavelength, bandwidth, and energy strongly affect bacterial activity.

In the present study, the expression of genes related to FimA fimbriae formation (*fimA, fimC*, and *fimD*) was also reduced after the irradiation of 450-nm LED light. To the best of our knowledge, this is the first study to examine the effects of blue LED irradiation without photosensitizers on FimA fimbriae genes. FimA is one of the main fimbriae of *P. gingivalis* and mediates bacterial colonization and biofilm formation in periodontal tissues. It also induces periodontal tissue inflammation through various pathways [42,43]. As mentioned above, the present study showed the reduction of gene expressions related to gingipains and hemin binding protein 35 (HBP35), which are involved in the formation and maturation of fimbriae [44]. Collectively, our results suggested that blue LED irradiation inhibits FimA fimbriae formation and attenuates bacterial activity.

LED irradiation at 625 nm increased the expression of genes related to DNA replication (*polA*) and homeostasis by degrading and removing abnormal membrane proteins (*ftsH*). These results suggest that the bacteria may recover from LED-induced damage and that red LED irradiation promotes these responses. While the expression of FimA-related genes tended to decrease, the present findings suggest that red light possibly alters bacterial pathogenicity without a bactericidal effect.

The present study showed that the effects of LED light on *P. gingivalis* and HGF-1 varied depending on the wavelength. Blue (450 and 470 nm) light showed antibacterial effects without a significant influence on the viability of HGF-1, whereas yellow-green (565 nm) and red (625 nm) light induced HGF-1 proliferation, which may promote periodontal tissue healing. These results show that the application of light with a combination of different wavelengths (*i.e.*, a combination of 450- and 625-nm LEDs) might be a novel method to achieve antibacterial effects and simultaneously promote periodontal tissue healing in clinical practice. A limitation of this study is that although altered expression of genes related to the pathogenesis of *P. gingivalis* was observed following blue LED irradiation, we have not yet verified the changes in the characteristics of this bacterium. In addition, we evaluated the effect of LED light only on *P. gingivalis* in this study. Indeed, *P. gingivalis* is a representative periodontopathic bacterium and a keystone pathogen that causes dysbiosis of the periodontal microbiome [45]; however, the oral cavity represents one of the largest microbiome in the human body, with more than 700 bacterial species [46]. The antibacterial effects and phototoxicity of LEDs against periodontal bacteria would vary depending on the bacteria because of different levels of endogenous photosensitizers. We plan to clarify these effects on multiple microorganisms in the future.

5. Conclusion

The effects of LED light on *P. gingivalis* and HGF-1 varied with the wavelength under the same irradiation conditions. When irradiated at 27 J/cm^2 (150 mW/cm^2, 3 min), UVA light (365 nm) showed the highest antibacterial effect and the highest cytotoxicity on HGFs. In contrast, blue light (450 and 470 nm) showed high antibacterial activity with low cytotoxicity, and using these wavelengths under the present irradiation conditions is promising for the elimination of *P. gingivalis* in periodontal therapy. In addition, blue light may attenuate the pathogenesis of *P. gingivalis*. Although yellow-green (565 nm) and red (625 nm) LEDs exerted no antibacterial effect, they may promote wound healing.

CRediT authorship contribution statement

Sakura Hayashi: Writing – original draft, Investigation, Funding acquisition, Formal analysis. **Yasuo Takeuchi:** Writing – review & editing, Supervision, Conceptualization. **Koichi Hiratsuka:** Writing – review & editing, Visualization. **Yutaro Kitanaka:** Investigation. **Keita Toyoshima:** Investigation. **Takashi Nemoto:** Investigation. **Nay Aung:** Investigation. **Masahiro Hakariya:** Writing – original draft, Investigation. **Yuichi Ikeda:** Writing – review & editing. **Takanori Iwata:** Writing – review & editing. **Akira Aoki:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare no conflicts of interest associated with this manuscript.

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